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Comparative Study on Triclosan Interactions in Solution and in the Solid State with Natural and Chemically Modified Cyclodextrins

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Abstract

The aim of this study was to investigate the interactions of triclosan (TRI), a poorly water-soluble antimicrobial drug, with natural crystalline cyclodextrins (α-, β- and γ-Cd) and the corresponding hydroxypropylated amorphous derivatives (HPα-, HPβ-, and HPγ-Cd) and evaluate their effectiveness as complexing and solubilizing agents towards the drug. Equimolar solid systems were prepared using different techniques (physical mixing (PM), kneading (KN) and coevaporation (COE)) in order to evaluate the influence of the preparation method on the performance of the end products. Drug–carrier interactions were investigated both in aqueous solution, using phase-solubility analysis, fluorescence and circular dichroism (CD) techniques, and in the solid state, using differential scanning calorimetry (DSC) supported by thermogravimetric analysis (TGA), X-ray powder diffractometry (XRPD) and scanning electron microscopy (SEM) analysis. Among the native cyclodextrins, β-Cd seemed to have the most suitable cavity to fit the drug molecule, whereas the α-Cd cavity was too small and the γ-Cd cavity too large to establish stable interactions with the guest. However, due to the B_S-type phase solubility diagram, its solubilizing efficiency was very limited. The presence of the hydroxypropylic substituents improved, in all cases, Cd solubilizing and complexing efficacies towards the drug. This was particularly evident in the case of HPγ-Cd, whose stability constant was about 200-fold higher than that of the native γ-Cd. HPβ-Cd was the most effective carrier for TRI, showing a solubilizing power about 20 times higher than the corresponding native Cd and about 2-fold that of the other hydroxypropyl derivatives. Moreover, a clear influence of the preparation method on the properties of the final products was observed. The COE method with hydroxypropylated cyclodextrins seemed the most suitable technique in achieving the complete drug amorphization and/or inclusion complexation.

Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a broad-spectrum antibacterial and antimicrobial agent. As a result of its bacteriostatic activity against a wide range of both gram-positive and gram-negative bacteria [1], it has found increasing and wide use in personal care products, such as toothpastes, deodorant soaps, deodorants, antiperspirants and body washes, cosmetic and antimicrobial creams and lotions [2]. In particular, the presence of antibacterial agents in products intended for oral care is an efficient tool against bacterial plaque formation and consequent gingivitis [3, 4]. The anti-plaque effect depends not only on the antimicrobial power of the agent, but also on both its ability to dissolve in the saliva and its affinity for the lipophilic sites of the oral cavity [5]. However, the very low water-solubility of triclosan (TRI) (only about 10 $\mu g\ ml^{-1}$) can give rise to formulation problems and can also reduce and make variable its biological activity. For these reasons, different attempts to improve the aqueous solubility of triclosan, such as micellar solubilization, complexation and salt formation, have been made in recent years [6, 7].

Among the approaches aimed at enhancing the solubility and dissolution properties of hydrophobic drugs, cyclodextrin complexation is known as one of the most effective methods [8, 9]. Cyclodextrins are well-known cyclic oligosaccharides with a lipophilic central cavity and a hydrophilic outer surface able to form inclusion complexes with lipophilic molecules [10]. Their effectiveness toward drugs can be strongly affected by several factors, such as the host cavity size, the presence and type of substituents on the macrocyclic ring, as well as the method used for the complex preparation [11–15].

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Therefore, in the present study we evaluated and compared the complexing power and solubilizing efficiency of the most common native crystalline cyclodextrins (α-, β- and γ-Cd) and their corresponding amorphous hydroxypropyl derivatives towards TRI, investigating the role of the carrier cavity size, the presence or not of the substituents on the ring, the crystalline or amorphous state of cyclodextrins and the drug–carrier system preparation method (physical mixing (PM), kneading (KN), coevaporation (COE)).

Drug-carrier interactions in aqueous solutions were studied by phase-solubility analysis and fluorescence and circular dichroism spectroscopies, while differential scanning calorimetry, supported by thermogravimetry (TG), X-ray powder diffractometry (XRPD) and scanning electron microscopy (SEM), analyses, were employed to characterize the solid-state of all drug–cyclodextrin equimolar solid combinations.

Experimental methods

Materials

Triclosan (TRI) was a gift from Comercial Química Massó (Barcelona, Spain); native cyclodextrins (α-Cd, β-Cd, and γ-Cd) were from Sigma (St. Louis, USA); hydroxypropyl derivatives (HPα-Cd, HPβ-Cd, and HPγ-Cd) with MS – 0.6 were kindly provided by Wacker-Chemie GmbH (München, Germany). All other reagents were of analytical reagent grade.

Preparation of binary systems

Equimolar (1:1 mol/mol) physical mixtures of TRI with each Cd were prepared by homogeneous mixing for 15 min of previously sieved (granulometric fraction smaller than 100 μm) and weighed powders. Kneaded systems were prepared by adding a small volume of ethanol to the corresponding physical mixtures and kneading the resultant systems until a homogeneous slurry was obtained; the products were then dried at 40 °C for 48 h and sieved before using (granulometric fractions smaller than 100 μm were collected for further studies). Coevaporated products were obtained by dissolving the corresponding blends in water-ethanol (50/50 v/v) solutions and then removing the solvent using a rotary evaporator. The resulting solid mass was then sieved and the granulometric fraction smaller than 100 μm was collected.

Phase solubility studies

Phase solubility studies of TRI were performed by adding excess amounts of drug (50 mg) to 10 ml of water or aqueous solutions of Cd in the 0-25 mM concentration range (0–13.0 mM in the case of β-Cd) in sealed glass containers. The suspensions were equilibrated upon magnetically stirring at constant temperature (25 ± 0.5 °C) for 72 h. Aliquots were then withdrawn, filtered through a 0.45 μm membrane filter and spectrophotometrically assayed for drug concentration at λ_{max} = 280 nm (Perkin-Elmer 552S spectrophotometer). Each experiment was performed in triplicate (CV < 2%). The drug–Cd stability constants were calculated from the slopes of the linear portions of the respective phase-solubility diagrams [16].

Fluorescence spectra

Fluorescence measurements were carried out using a Perkin-Elmer mod. 650-10S spectrofluorimeter. The fluorescence intensities of the 0.04 mM TRI unbuffered aqueous solution in the absence and in the presence of different concentrations of each Cd (3, 6 and 9 mM) were measured at excitation and emission maxima of 325 and 365 nm, respectively.

Circular dichroism spectra (CDS)

The circular dichroism spectra (CDS) were obtained using a Jusco J-500D spectropolarimeter (time constant, 16; chart speed, 10 cm min^{-1}; sensitivity, 0.5 m cm^{-1}; wavelength expansion, 10 nm cm^{-1}; scan speed, 50 cm min^{-1}; range, 350 220 nm) on 0.03 mM unbuffered aqueous solutions of TRI in the absence and in the presence of each Cd at a concentration of 3, 6 and 9 mM.

Differential scanning calorimetry (DSC)

DSC analyses were recorded on a Mettler TA 3000 differential scanning calorimeter equipped with a DSC 20 cell. Samples of 5–10 mg (Mettler-M3 microbalance) were scanned at a heating rate of 10 °C min^{-1} in pierced aluminium pans in the 30–350 °C temperature range under static air. The instrument was calibrated with a standard sample of indium.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was carried out using a Mettler TA 3000 apparatus equipped with a TG50 cell on 7–10 mg samples in open alumina crucibles. Samples were analysed at the heating rate of 10 °C min^{-1} over the 30–600 °C temperature range.

X-ray powder diffractometry (XRPD)

X-ray powder diffraction patterns were obtained using a computer-controlled Philips XPert apparatus over the 5–40° 2θ range at a scan rate of 1° min^{-1}. Samples were irradiated with monochromatic CuK_α radiation. The voltage, current and time per step used were 40 kV, 55 mA and 1 s, respectively (C.A.I., DRX, U.C.M.).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used as support technique for the solid state characterization. Analyses were performed using a Philips XL-30 scanning electron microscope. Prior to examination, samples were gold sputter-coated to render them electrically conductive.

Results and discussion

Characterization in solution

Figure 1 shows the phase-solubility diagrams in aqueous solutions at 25 °C of TRI in the presence of the different natural and derivative Cds. In the case of the natural Cds (Figure 1a), different behaviours can be observed. In fact in the presence of α-Cd, the TRI concentration showed a linear trend, slightly increasing with increasing ligand concentration, revealing a Higuchi A_L-type curve [16]. A B_s-type diagram [16] was instead obtained in the presence of β-Cd, showing an initial rising portion followed by a plateau region and then by a decrease, indicative of the precipitation of a poorly soluble complex at high β-Cd concentration. The stability constant of the complex was determined in the initial linear range (0–7 mM Cd). Finally, in the presence of γ-Cd, a very small increase in drug solubility was observed with increasing Cd concentration, implying the absence of valuable interactions in solution between TRI and γ-Cd. A_L diagrams, indicative of the formation of 1:1 soluble complexes [16] were observed with all hydroxypropylated Cds (Figure 1b). As it is evident, the presence of the hydroxypropyl substituents was in all cases favourable in improving the Cd solubilizing and complexing properties toward the drug with respect to the corresponding native ones. In particular, the better solubilizing efficiency towards TRI shown by HPβ-Cd in comparison with the natural one was in accord with the findings of Loftsson [6], whereas it differed from those of Grove [7], who, instead, obtained a similar performance with both carriers.

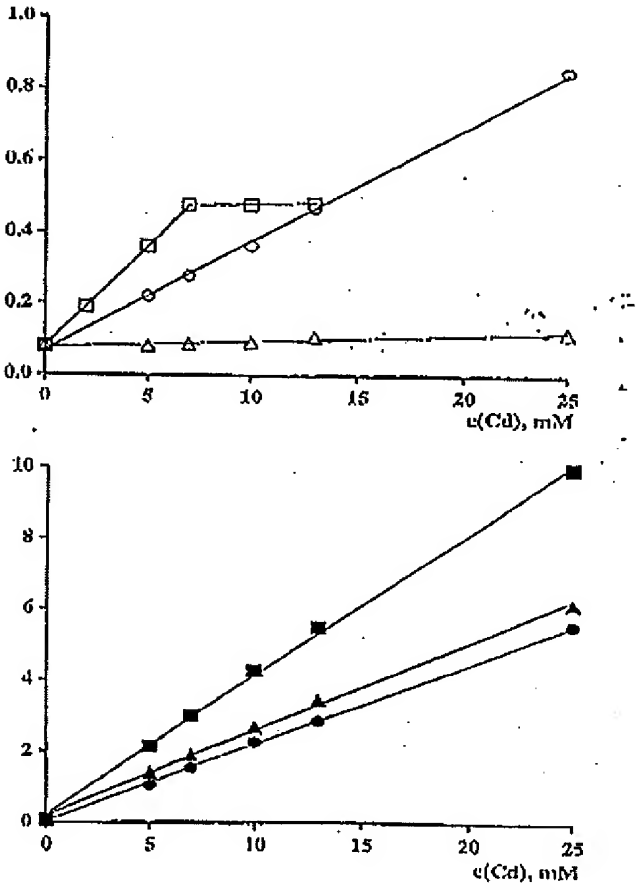

Figure 1. Phase-solubility diagrams in aqueous solutions at 25 ± 0.5 °C of TRI in the presence of natural (a) or hydroxypropyl derivative (b) cyclodextrins. Key (a): α-Cd (◇), β-Cd (□), γ-Cd (△); (b): HPα-Cd (♦), HPβ-Cd (■), HPγ-Cd (▲).

The apparent 1:1 stability constant values of TRI with each Cd, obtained from the linear portion of the corresponding phase-solubility diagrams and the relative increases in TRI solubility at 25 °C are shown in Table 1. Among the native Cds, β-Cd clearly showed the highest stability constant value, followed by α-Cd, while a very low value was obtained for γ-Cd. The results of these studies seem to indicate that β-Cd has the most suitable cavity for hosting the TRI molecule. In fact, the α-Cd cavity may be too small to suitably fit the guest, thus resulting in a lower complexing power towards the drug than β-Cd. On the other hand, the excessively large cavity of γ-Cd did not allow establishment of sufficiently strong interactions with the included guest molecule.

The more elevated values of the stability constants obtained for derivative Cds in comparison with the corresponding parent ones has been attributed to the extended hydrophobic region of the Cd cavity due to the presence of the hydroxy–alkylic groups which crown the ring edge [17]. The important role of the

Table 1. Stability constants for the interaction of TRI with native and hydroxypropylated cyclodextrins in water at 25 °C

Cyclodextrin	$K_{1:1}$ (M^{-1})	Solubilizing efficiency[a]
α-Cd	390	10.5
β-Cd	750	6.0
γ-Cd	20	1.3
HPα-Cd	3500	68.5
HPβ-Cd	8100	123.2
HPγ-Cd	3970	74.2

[a] Relative TRI solubility increase at 25 °C (ratio between drug solubility in 25 mM aqueous solution of Cd or 13 mM β-Cd and in water).

hydroxypropyl substituents in stabilizing the TRI inclusion complexation was particularly evident in the case of the complex with HPγ-Cd, whose stability constant was about 200-fold higher than that of the native γ-Cd and also slightly higher than that of HPα-Cd. Evidently, in this case the presence of the substituents allowed formation of stronger interactions with the guest molecule, stabilizing its inclusion into the host cavity. HPβ-Cd was the most effective partner for TRI, allowing a 120-times increase of the pure drug aqueous solubility and showing a solubilizing efficiency about 20 times higher than the parent Cd and about two times of that of the other derivatives, which, on the contrary, revealed similar solubilizing efficiencies.

Fluorescence studies (Figure 2) corroborated these findings. A direct relationship has been found between the intensity of the drug fluorescence changes in the presence of Cds and the strength of binding between the two components. In fact, TRI fluorescence slightly increased in the presence of α-Cd, whereas a more evident increase was observed in the presence of β-Cd; on the contrary, no valuable modifications in the TRI fluorescence signal were registered in the presence of γ-Cd, thus confirming the almost total lack of interaction in solution of the drug with such Cd (Figure 2a). In the presence of Cd derivatives, the enhancement of TRI fluorescence was more marked than that observed with the corresponding native ones under the same conditions. In particular, a more than 30-fold fluorescence intensity increase was registered in the presence of 9 mM HPβ-Cd compared with the pure drug solution. Moreover, the fluorescence intensity increase obtained in the presence of HPγ-Cd was comparable to that obtained for HPα-Cd.

Figure 3 shows the TRI circular dichroism spectra in the absence and presence of α-, and β-Cd. Interactions in solution of TRI with both Cds caused significant changes in the drug spectrum, as a consequence of its inclusion complexation into the host cavity. The negative absorption maximum at 278 nm shown by TRI alone, was shifted to higher wavelengths with a concomitant decrease in intensity in the presence of α-Cd. Addition of β-Cd caused even more marked changes in the drug spectrum, which showed an inversion of the ellipticity sign, presenting two positive maxima at 275 and 290 nm. These spectral differences, and in particular the different sign of optical activity may be mainly ascribed to the different orientation and/or disposition of the guest molecule within the cavities of the two Cds. On the other hand, no appreciable changes in the TRI spectral pattern were observed in the presence of γ-Cd, thus further confirming the nearly total absence of interactions with such carrier.

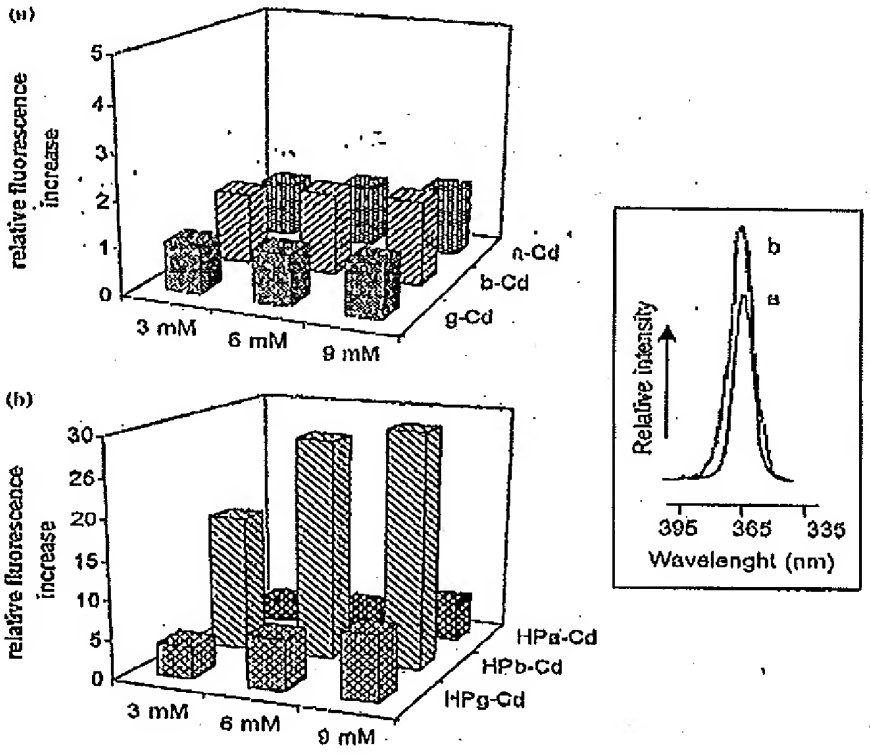

Figure 2. Relative fluorescence increase of 0.4 mM triclosan (TRI) aqueous solution in the presence of increasing concentrations of natural (a) or hydroxypropyl derivative (b) cyclodextrins. The fluorescence spectra of TRI alone (a) and in the presence of 6mM α Cd (b) are shown in the window as an example.

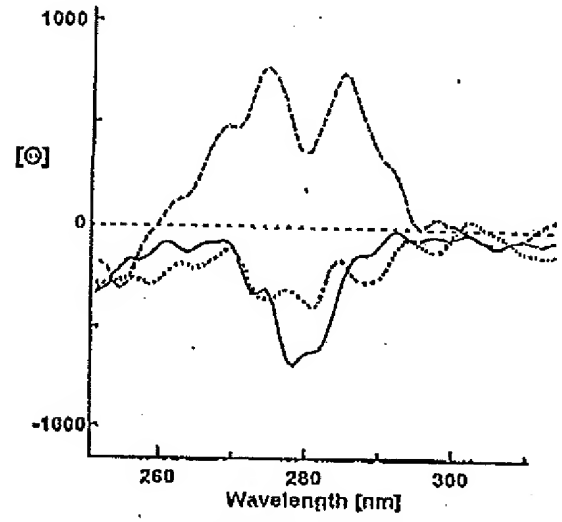

Figure 3. Circular dichroism absorption spectra of TRI alone (unbroken line) or in the presence of 6 mM α-Cd (dotted line) and β-Cd (broken line).

Solid state characterization

The thermal curves of some representative equimolar TRI–Cd solid systems are shown in Figure 4. DSC profiles of pure drug and Cds are reported for reference purposes. The DSC curve of TRI exhibited a sharp endotherm peaked at 57.9 °C with a fusion enthalpy of 61.3 J g^{-1} corresponding to the melting process of the anhydrous crystalline drug, followed by a broad endothermic effect at about 300 °C due to decomposition phenomena. The DSC curve of β-Cd displayed a single intense band at 100 °C whereas a broad endothermic effect between 60 and 120 °C was registered for the amorphous HPβ-Cd, indicating in both cases dehydration processes. DSC curves of both physical mixtures showed similar profiles, with the characteristic thermal behaviour of both components still detectable in all cases. The presence of the TRI melting peak also in the thermal curves of kneaded products with both Cds (even though markedly reduced in its intensity with respect to the physical mixtures) might indicate that the drug molecule was not completely included in the apolar cavity of Cd during the kneading process. On the contrary, the total disappearance of the TRI fusion peak was observed for both coevaporated products, thus indicating drug amorphization and/or complexation as a consequence of a more intimate physical contact between the components due to the preparation method.

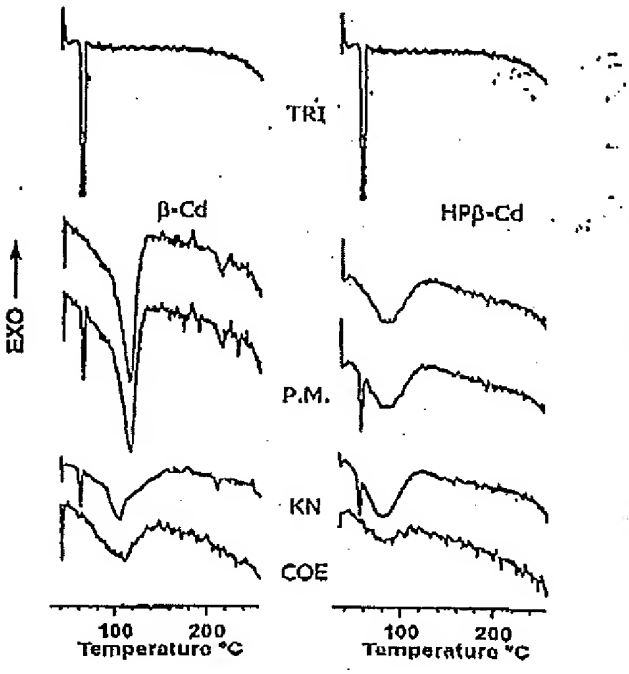

Figure 4. DSC curves of pure TRI, β-Cd, and HPβ-Cd, and their equimolar physical mixtures PM, kneaded KN and coevaporated COE products.

Unfortunately, TGA was not particularly useful for further characterization of drug–Cd solid systems. In fact, for each series of binary systems, no appreciable differences in the mass loss behaviour between the simple physical mixture and the other elaborated products were observed.

Figure 5 shows the X-ray powder diffraction patterns of pure drug and selected Cds, and their simple blends, kneaded and coevaporated products. The diffraction pattern of TRI displayed several sharp peaks, indicative of its crystalline nature. A typical crystalline pattern was shown by β-Cd, whereas a diffuse pattern was obtained from the amorphous derivative.

Some drug crystallinity loss can be noted in physical mixtures with both Cds. The characteristic peaks of both components, even if markedly reduced in intensity, are still detectable in both kneaded and coevaporated systems with β-Cd, thus indicating that the drug maintained a residual crystallinity in these products as well. Therefore, the total disappearance of drug fusion peak revealed by DSC analysis for the coevaporated product could be a consequence of drug–carrier interactions induced by the thermal energy supplied to the system during the scan. No significant differences were observed between the simple blend and the kneaded system with HPβ-Cd, in which the drug maintained an almost crystalline pattern. On the contrary, the diffuse pattern obtained for the coevaporated system with HPβ-Cd indicated the complete drug amorphization as a consequence of the homogenous dispersion of TRI in the amorphous carrier induced by coevaporation.

Finally, SEM analyses were carried out to investigate the morphologies of pure components and their combinations. Some selected representative images are

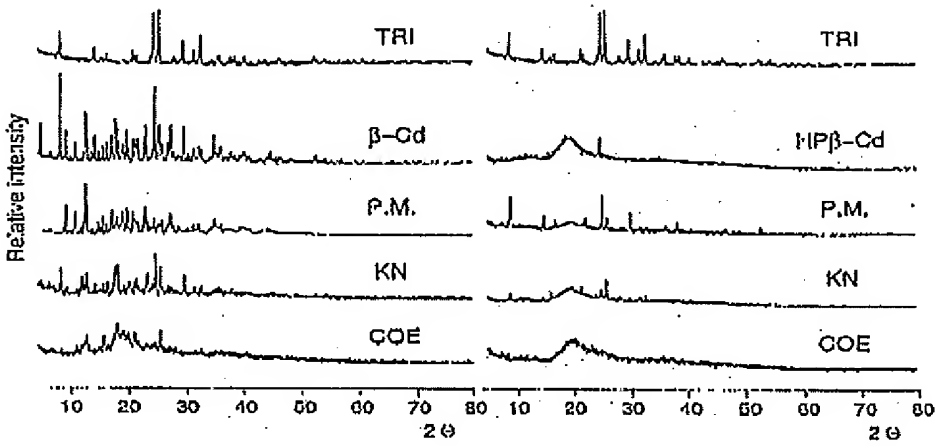

Figure 5. X-ray powder diffraction patterns of pure TRI, β-Cd, and HPβ-Cd, and their equimolar PM, KN and COE products.

reported in Figure 6. Individual stick-like crystals with smooth surfaces were observed for TRI, whereas spherical particles of various size with some concavities on the surface were revealed for HPβ-Cd. The results of SEM analysis of the different drug-Cd combinations were consistent with DSC and X-ray findings. In fact, it was possible to clearly identify and differentiate the two components in all the physical mixtures. By contrast, as a consequence of the preparation method, significant morphological changes occurred in coevaporated systems which appeared as amorphous agglomerates of glassy homogeneous flakes, and where only very few and small smooth needles of TRI were adhered to the surface.

Conclusions

Solution studies have shown that the cavity size of the host is decisive for an optimum fit between the Cd cavity and the TRI molecule. The observed rank order for the complexing ability is β-Cd ≫α-Cd ≫γ-Cd. The higher affinity of TRI towards β-Cd can be accounted by a deeper, more complete and better fitting inclusion of the drug. By contrast, the α-Cd cavity seems to be too small to suitably fit the guest, and the γ-Cd cavity too large for stably including the guest molecule.

On the other hand, the inclusion behaviour of TRI with the hydroxypropyl derivatives highlights the important role of the substituent in determining

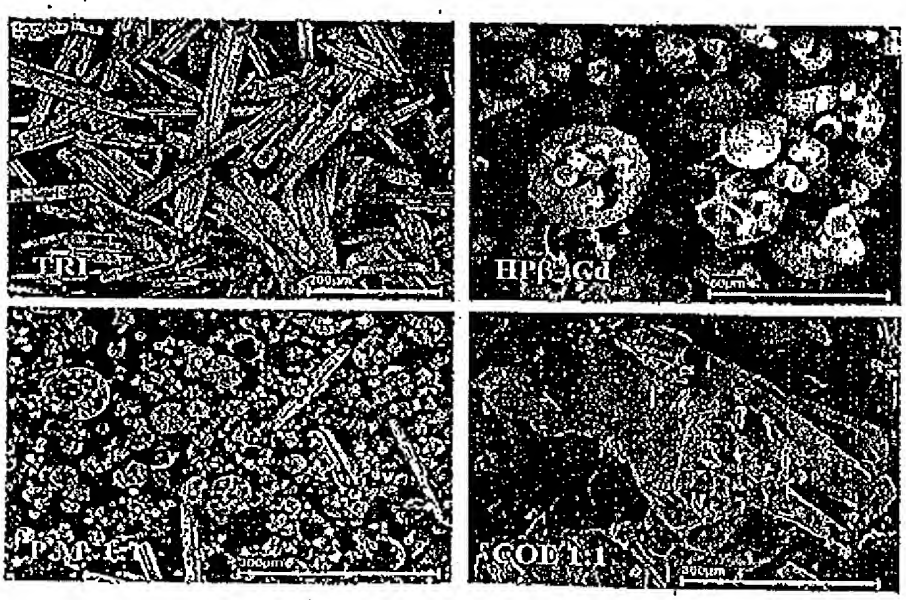

Figure 6. SEM micrographs of pure TRI, HPβ-Cd and their PM, KN and coevaporated (COE) products.

the Cd complexing and solubilizing capacities toward the drug. The greater effectiveness exhibited by the Cd derivatives in comparison with the corresponding natural ones can be ascribed to their higher water solubility and to the extended hydrophobic cavity, due to the presence of the hydroxyalkyl derivatives on the edge of the ring. This effect is particularly evident in the case of HPy-Cd, which, conversely to the parent compound, allowed the formation of a stable complex. In fact, a variation of the rank order of stability constant values was found: HPβ-Cd $\gg$ HPy-Cd $\geq$ HPα-Cd.

The β-Cd-derivative was the best partner for the drug, showing the highest complexing power and solubilizing ability among all the examined carriers.

Finally, a clear influence of the preparation method on the characteristics of the final solid products was observed. In particular, coevaporation seems to be the most effective technique, allowing a more intimate contact, and therefore better interactions between the components, with a consequent complete drug amorphization and/or inclusion in the carrier.

Acknowledgements

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